Chapter 4
Low-Cost DNA Extraction

Abstract The methods described in this chapter were developed to avoid toxic organic phase separation utilized in many low-cost DNA extraction protocols such as the CTAB method. The steps involve: (1) lysis of the plant material, (2) binding of DNA to silica powder under chaotropic conditions, (3) washing the bound DNA, and (4) elution of DNA from the silica powder. This method has been tested in several plant species and the applicability of such DNA preparations for molecular marker studies in barley is shown in Chap. 8.

4.1 Materials

Chemicals, enzymes, and equipment are listed in Table 4.1, and working stocks in Table 4.2.

4.2 Methods

4.2.1 Preparation of Silica Powder DNA Binding Solution

1. Transfer silica powder (Celite 545 silica) into a 50-ml conical tube (fill to the 2.5 ml line = approximately 800 mg).
2. Add 30 ml dH_2O.
3. Shake vigorously (vortex and invert 15 times or until a hydrated slurry forms).
4. Let the slurry settle for approximately 15 min.
5. Remove (pipette off) the liquid.
6. Repeat steps 2–5 an additional two times for a total of three washes. After the last washing step suspend the hydrated silica in a volume of water equal to the volume of silica (typically up to the 5-ml mark on the conical tube). This is the liquid silica stock (LSS) and can be stored at RT for up to 1 month.
7. Prior to use, suspend LSS by vortexing for 30 s or until a homogenized slurry is formed. Transfer 50 µl into 2-ml tubes. Prepare one tube per tissue sample.

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Table 4.1 Chemicals, enzymes and equipment for low-cost DNA extraction

Material description	Examples of suppliers and catalogue numbers
Celite 545 silica powder (Celite 545-AW reagent grade)	Sigma 20199-U
SDS (sodium dodecyl sulfate)	Sigma L-4390
50-ml conical tube with cap	Fisher Scientific 14-432-22
Sodium acetate anhydrous	Sigma S-2889
NaCl (sodium chloride)	Sigma S-3014
RNase A	Sigma R6513
Ethanol (absolute)	Fisher Scientific BP2818-4
H_2O (distilled or deionized and autoclaved)	
Potassium iodide	Sigma P2963
Guanidine thiocyanate (optional)	Sigma G9277
Microcentrifuge tubes (1.5 and 2.0 ml)	Any general laboratory supplier
Micropipettes (1,000, 200, and 20 µl)	Any general laboratory supplier
Microcentrifuge	Eppendorf Centrifuge 5415D
Vortex mixer	Vortex Genie 2, Fisher Scientific NC9864336
Metal beads (tungsten carbide beads, 3 mm)	Qiagen 69997
Sea sand (optional)	Sigma 274739
Agarose gel equipment	Horizontal electrophoresis from any general laboratory supplier

ATTENTION: try to keep the silica suspended when transferring to tubes to ensure that all tubes receive the same amount of LSS.

8. Add 1 ml H_2O per tube to perform a final wash step.
9. Mix by vortexing for 15 s or until silica is fully suspended.
10. Centrifuge at full speed ($16{,}000 \times g$) for 20 s.
11. Pipette off the liquid.
12. Add 700 µl DNA binding buffer (6 M potassium iodide or alternatively 6 M guanidine thiocyanate).
13. Suspend the silica in DNA binding buffer by vortexing for 15 s.
14. The Silica Binding Solution (SBS) is now ready for use.

4.2.2 Low-Cost Extraction of Genomic DNA

1. Prepare an ice bath.
2. Label 2-ml tubes containing three metal tungsten carbide beads with sample names.
3. Add the dried tissue to the appropriate tube.
4. Tape the tubes onto a vortex mixer (Fig. 4.1) and vortex on high setting for 30 s or until the material is ground to a fine powder. NOTE: If the tissue is not fully ground, grinding is facilitated by addition of 0.2 g of purified sea sand per tube. It is common for some tissues to not be completely ground to a powder. High

Table 4.2 Working stocks for DNA extraction

Stock solution	Recipe	Comments
5 M NaCl	MW = 58.44 g/mol 29.22 g/100 ml	Do not use if precipitate forms. Either heat to get fully back into solution or discard and make fresh
3 M Sodium Acetate (pH 5.2)	MW = 82.03 g/mol 24.61 g/100 ml	Adjust pH value with glacial acetic acid
95 % (v/v) Ethanol	95 ml ethanol abs 5 ml H_2O	Use fresh. Ethanol absorbs water and the % will drop over time
Tris-EDTA (TE) buffer (10×)	100 mM Tris-HCl, pH 8.0 10 mM EDTA	Tris and EDTA can be prepared from powders. This may be less costly. However, note that the pH of Tris changes with temperature
Lysis buffer	0.5 % SDS (w/v) in 10× TE 0.5 g SDS/100 ml	
DNA binding buffer	6 M Potassium Iodide (KI) Alternative: 6 M Guanidine thiocyanate	ATTENTION! It takes several hours until fully dissolved (leave it for approximately 4–5 h)
Wash buffer	1 ml of 5 M NaCl 99 ml of 95 % EtOH	ATTENTION! Prepare fresh because the salt precipitates during storage
DNA elution buffer	1x TE-buffer	Tris-EDTA buffer is advised for most applications. If the presence of EDTA is inhibitory to downstream applications, elution can be carried out using 10 mM Tris

Fig. 4.1 Sample grinding is accomplished by combining desiccated leaf material and metal beads into a 2-ml tube (*left panel*) and taping sample tubes to a standard vortex mixer (*middle panel*). Grinding is complete when a find powder is produced. The presence of unground tissue with the powder does not affect the quality of extracted DNA (*right panel*, and example data in Chap. 8)

quality DNA can still be produced from such samples. Sample degradation can occur after prolonged vortexing. It is therefore suggested to test different grinding times to find proper conditions to maximize both genomic DNA yield and quality.

5. Add 800 µl of Lysis Buffer and 4 µl RNAse A (10 µg/ml) to each tube. NOTE: See Sect. 4.3 for alternative buffers.

6. Vortex at a high speed for approximately 2 min until the powder is fully hydrated and mixed with buffer.
7. Incubate for 10 min at RT.
8. Add 200 µl 3 M sodium acetate (pH 5.2). Mix by the inversion of tubes and incubate on ice for 5 min.
9. Centrifuge at 16,000×*g* for 5 min at RT to pellet the leaf material.
10. Label the tubes with aliquots of silica binding solution (SBS, 700 µl) with the sample name.
11. Transfer the liquid into appropriately labelled SBS-containing tubes. DO NOT TRANSFER THE LEAF MATERIAL!
12. Completely suspend the silica powder by vortexing and inverting the tubes (approximately 20 s).
13. Incubate for 15 min at RT (on a shaker at 400 rpm, or invert tubes every 3 min by hand).
14. Centrifuge at 16,000×*g* for 3 min at RT to pellet the silica.
15. Remove the supernatant with a pipette and discard (the DNA is bound to the silica at this stage).
16. Add 500 µl of freshly prepared wash buffer to each tube.
17. Completely suspend the silica powder by vortexing or inverting the tubes (approximately 20 s).
18. Centrifuge at 16,000×*g* for 3 min at RT to pellet the silica. Remove the supernatant and keep the pellet.
19. Repeat steps 16–18.
20. Centrifuge the pellet for 30 s and remove any residual wash buffer with a pipette.
21. Open the lid on tubes containing silica pellet and place in a fume hood for 30 min to fully dry the pellets (NOTE: This can be done for a longer period on the bench top if a fume hood is not available).
22. Add 200 µl TE buffer to each tube to elute the DNA. The DNA is now in the liquid buffer. A buffered solution is preferred over water to prevent degradation.
23. Completely suspend the silica powder by vortexing and inversion of tubes (approximately 20 s).
24. Incubate at RT for 5 min.
25. Centrifuge at 16,000×*g* for 5 min at RT to pellet the silica.
26. Label new 1.5-ml tubes with sample numbers/codes.
27. Collect the liquid containing genomic DNA and place into new tubes.
28. Store DNA temporarily at 4 °C before checking the quality and quantity.
29. Evaluate the quality and quantity of the extracted DNA. While fluorometric and spectrophotometric methods have their advantages, it is suggested that samples are evaluated using agarose gel electrophoresis and a quantitative marker so that sample degradation and the presence of any RNA can be monitored. See Chap. 8 for example data.

Table 4.3 Alternative lysis buffers for DNA extraction

Lysis buffer (LB) name	Recipe (in 10× TE)	Preparation in final volume of 100 ml in 10× TE
LB1	0.5 % SDS (w/v)	0.5 g SDS
LB2	0.5 % SDS (w/v)	0.5 g SDS
	0.5 M NaCl	10 ml of 5 M NaCl
	3 % PVP (w/v)	3 g
LB3	0.5 % SDS (w/v)	0.5 g SDS
	0.5 M NaCl	10 ml of 5 M NaCl
	3 % PVP (w/v)	3 g
	1 % sodium sulfite	1 g
LB4	0.5 % SDS (w/v)	0.5 g SDS
	0.5 M NaCl	10 ml of 5 M NaCl
	3 % PVP (w/v)	3 g
	1 % sodium sulfite	1 g
	2 % N-lauryl-sarcosyl sodium salt	2 g

4.3 Alternative Buffers for DNA Extraction

The main areas for the optimization of DNA-extraction methods include increasing sample yield, reducing co-purification of unwanted components (e.g., polysaccharides, and polyphenols), and reducing sample degradation. To a large extent, providing the starting tissues are of good quality, all three areas can be influenced by the sample lysis procedure. Table 4.3 lists four lysis buffers to optimize the isolation of DNA from grapevine and sorghum. A more thorough compilation of buffer components and additives to enhance DNA isolation in the presence of secondary compounds can be found in Weising et al. (2005). Data from buffer optimizations are shown in Chap. 8.

Reference

Weising K, Nybom H, Wolff K, Kahl G (2005) DNA fingerprinting in plants: principles, methods and applications. CRC Press, Boca Raton, FL